201-14424

Peter **Wendolkowski**
04/30/2003 03:05 PM

To: Peter Wendolkowski/DC/USEPA/US@EPA
cc:
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Subject: Public comments on PPG's test plan for propanoic acid

Jessica Sandler <jessicas@peta.org> on 04/25/2003 05:04:39 PM

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Subject: Public comments on PPG's test plan for propanoic acid

Attached please find the comments of the American animal protection community on PPG's HPV test plan for propanoic acid. These comments are in follow-up to our January 2 letter to Stephen Johnson regarding this test plan and the manner in which it was handled by the EPA (also attached). We continue to have major concerns about both PPG's testing proposals and the EPA's failure to properly review test plans per the October 1999 agreement to reduce the number of animals killed in this program.

PPG's revised test plan still fails to provide basic information needed to properly evaluate the testing proposals. We ask, again, that the EPA take its review of test plans seriously, and reject a plan that is clearly inadequate.

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PETA **letter to EPA on PPG.pdf** **HPV test plan comments -- PPG propanoic** acid2.pdf

April 25, 2003

Christine Todd Whitman, Administrator
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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Subject: Comments on PPG's revised HPV test plan for propanoic acid, 2-hydroxy-compound with 3-[2-(dimethylamino)ethyl]1-(2-ethylhexyl)(4-methyl-1,3-phenylene)bis[carbamate](1:1)

Dear Administrator Whitman:

The following are comments on the revised test plan for propanoic acid, 2-hydroxy-, compound with 3-[2-(dimethylamino)ethyl]1-(2-ethylhexyl)(4-methyl-1,3-phenylene)bis[carbamate] (1:1), CAS no. 68227-46-3, submitted by PPG Industries, Inc. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal and environmental protection organizations have a combined membership of more than ten million Americans.

The first version of the test plan, an utterly inadequate document consisting of only a single one-page table, was posted in September 2002, with public comments due on January 17, 2003. The company that submitted the plan was listed as "confidential", even though the HPV program is considered to be a "public right to know" program. When PETA determined that PPG was, in fact, the company that had submitted this plan, PPG revoked its request for confidentiality. On January 2, 2003, PETA submitted brief comments (attached) to the EPA, pointing out that the test plan was inadequate, and stating that it was impossible to make substantive comments on it. In a telephone conversation with PETA on the same date, Dr. Barter of PPG stated that the original test plan submitted was "preliminary," and would be revised based on public comments received and "more details" provided at a later date.

A somewhat improved, although still highly inadequate, version of PPG's test plan was then posted on February 28, 2003, with a 60-day—rather than the usual 120-day—comment period. This test plan includes three animal tests, which will kill approximately 800 animals. Our criticisms of the revised plan center on the fact that it still provides insufficient information to allow for a reasonable public review. Some of the absent information should be readily available. For example, Dr. Barter, in the above-mentioned telephone conversation, stated that PPG does have a Material Safety Data Sheet for 68227-46-3, for use by PPG employees. However, we have been unable to obtain a copy of this document.

The following are examples of the inadequacies of this revised plan:

1. The test plan states that no experimentally determined physicochemical data are available (p. 5). In the telephone conversation referenced above, Dr. Barter informed us that PPG

has been using 68227-46-3 since at least 1990. If a company maintains that it has not obtained such basic data as solubility and vapor pressure for a compound to which its employees have been exposed for more than twelve years, this claim must be considered either untrue or indicative of an extremely negligent attitude. The vapor pressure and octanol/water partition coefficient have now been estimated, but no explanation is given as to why PPG has no plans to verify the estimates experimentally.

2. The test plan provides insufficient information about the form in which 68227-46-3 is manufactured, used, and transported. The test plan implies the existence of two solutions, as it states that 68227-46-3 is manufactured in the presence of 5% methyl isobutyl ketone, and is then diluted with 20% 2-butoxyethanol (p. 4). However, it gives no indication as to the other components of the two solutions, or the concentrations of any of the components. The test plan also does not state whether 68227-46-3 is ever used or transported in any form other than the two solutions mentioned. Without information about the compositions of these solutions, and any other forms to which exposure may occur, it is impossible to provide substantive comments or to critique the test plan in detail. Indeed, no testing should be proposed until such questions are answered. At the same time, it is difficult to understand why the planned tests are on a 71% aqueous *suspension* of 68227-46-3 (p. 4), rather than one of the solutions to which exposure can occur in a real-world context.

   As stated in the above paragraph, the compounds with which 68227-46-3 is known to occur are methyl isobutyl ketone (CAS no. 108-10-1) and 2-butoxyethanol (CAS no. 111-76-2). Methyl isobutyl ketone is used as a solvent in a wide range of industries, and its characteristics, including toxicity, have been extraordinarily well characterized: our searches of various databases show that more than a thousand toxicity reports have been published. It is toxic (WHO 1990), with a US permissible exposure limit in air of 100 ppm (29 CFR 1910.1000). 2-butoxyethanol is also widely used as a solvent and has significant toxicity ("Final report on the safety assessment of butoxyethanol" 1996), with a US permissible exposure limit in air of 50 ppm (29 CFR 1910.1000). Therefore, if the concentration of 68227-46-3 is low, the risk assessment of its solutions will be driven by the toxicity of the other components, and the toxicity of 68227-46-3 will be irrelevant. As no information is provided in the test plan about the relative concentrations of 68227-46-3, methyl isobutyl ketone and 2-butoxyethanol, it is premature to make any suggestions in this respect, and it inappropriate to propose testing until this issue is addressed.

3. In the case of a compound such as 68227-46-3, which is aromatic and ionic, with a variety of functional groups (amides, esters, alcohols), a considerable part of any toxicity is likely to be due to metabolites. PPG should therefore predict the principal metabolites from the structure of the compound, and assess the data available for them prior to proposing any additional testing on animals.

4. The test plan provides insufficient information about human exposure to 68227-46-3. It states that most of the 68227-46-3 manufactured is used as an intermediate (p. 4), but does not state whether it is a closed-system intermediate. It also provides no details about the transport of 68227-46-3 to the two companies to which it is sold, nor the numbers of workers exposed to it at those companies. Even if toxicity data were available, it would be

impossible to estimate the human and environmental risks due to 68227-46-3 in the context of this data vacuum. One of the most urgent tasks with respect to this compound is therefore to carry out an exposure assessment. An epidemiology study would also be appropriate.

For all the above reasons, it is impossible to properly critique the test plan. We therefore urge the EPA to reject this test plan in its entirety, and to request that PPG prepare a thoughtful and responsible test plan. The following comments, on the test plan as it stands, should therefore be regarded as merely provisional.

PPG plans to carry out an acute toxicity test (OECD guideline 425) and a combined repeat-dose/reproductive/developmental toxicity test (OECD guideline 422; test plan, p. 2), which will kill at least 685 mammals. However, it is highly premature to plan large-scale tests in the context of the current information vacuum. The data most urgently required at this stage are for exposure and *in vitro* toxicity and, without these basic data, mammalian toxicity data would have little or no value.

In addition to mammalian tests, PPG plans to carry out an acute fish toxicity test (OECD guideline 203; test plan, p. 2), which will kill 40-120 fish. However, the EPA has clearly stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of acute fish and *Daphnia* tests (EPA *Federal Register,* December 2000, p. 81695). The log $K_{o/w}$ value of 68227-46-3 has been calculated to be 4.38 (robust summaries, p. 1), and there is no plan to test this value experimentally (test plan, p. 5). Per the EPA's instructions, the fish test should therefore not be carried out. This is supported by the fact that the solubility is apparently so low that the planned tests have to use an aqueous suspension rather than solution (test plan p. 4).

An additional reason why the fish test is unnecessary relates to the purpose of the ecotoxicity tests. Fish tests are not intended to predict toxicity in individual fish, but to predict economic loss (to commercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The fish test therefore aims to show whether exposure to 68227-46-3 will result in large-scale fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. The toxicity of 68227-46-3 towards these types of organism is unknown, as shown by the inclusion in the test plan of tests on aquatic invertebrates and algae (p. 6). Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

Finally, if PPG does wish to carry out the tests indicated in the test plan, there is a range of *in vitro* and *in silico* alternatives to fish and acute and developmental mammalian toxicity tests, as detailed in the Appendix.

To conclude, PPG's revised test plan fails to provide basic information, the mammalian test plans are clearly premature, and the only firm conclusion that can be reached at this stage is that

the fish test is inappropriate. We call on the EPA to take its review of test plans seriously, and to reject a plan that is clearly inadequate. We remind the EPA of its commitment to a careful analysis of test plans with an eye towards reducing the number of animals killed wherever possible. While PPG mentions the October 1999 agreement with animal protection organizations in its testing proposal, its inadequate test plan will lead directly to the deaths of a large number of animals. Thus the spirit of the October 1999 agreement, as well as its specific requirements for thoughtful toxicology, are violated by this test plan.

Thank you for your attention to these comments. I can be reached at 757-622-7382, extension 1304, or via e-mail at JessicaS@PETA.org.

Sincerely,

Jessica Sandler, MHS
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Appendix: *In vitro* and *in silico* test methods

1. In silico *fish test substitute*. Quantitative structure activity relationship (QSAR) programs provide *in silico* methods for estimating toxicity to fish and other aquatic organisms. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002), for the HPV program.

2. In vitro *fish test substitutes*:

   (i) TETRATOX is an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997). With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997), and the extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

   (ii) The test protocol and performance parameters of the recently validated *Dar*T test are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, it uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

3. *Mammalian acute toxicity test substitute*. The test plan includes the following statement: "The cytotoxicity test could provide useful information to estimate starting doses for *in*

vivo acute toxicity testing" (p. 6). We welcome PPG's intention to use the *in vitro* cytotoxicity test as an adjunct, but we urge PPG to discuss with the EPA the possibility of using it instead of the *in vivo* test. In the Multicentre Evaluation of *In Vitro* Cytotoxicity, a worldwide study organized by the Scandinavian Society for Cell Toxicology, basal cytotoxicity assays were found to be more reliable predictors of human lethal doses, for 50 reference chemicals, than were rodent LD_{50} values (Clemedson 1996a, 1996b, 1998a, 1998b, 2000, Ekwall 1998a, 1998b, 2000). Furthermore, when certain other human toxicokinetic data, such as blood-brain barrier passage and timing of lethal action, were used in conjunction with the cytotoxicity results, the prediction of human lethal concentrations improved markedly (Ekwall 2000). The assay used involves measuring the effects of compounds on the viability of human basal keratinocytes, which is determined from the intensity of staining by neutral red, a dye that is taken up by healthy cells more than by dead and low-viability cells.

4. *Mammalian developmental toxicity test substitute*. *In vivo* developmental and reproductive toxicity tests have not been validated for humans. However, an *in vitro* embryotoxicity test method, the rodent embryonic stem cell test, has recently been validated by the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). If a positive result is found in the embryonic stem cell test, 68227-46-3 should be treated as a development toxicant/teratogen, and no further testing should be carried out within the screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than six months, we have received no reply. We urge PPG to correspond directly with the EPA on the incorporation of this validated non-animal test.

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